REMARKS

Entry of the instant amendment is respectfully requested. Applicants assert the amendment does not introduce new matter, does not raise new issues for the Examiner's consideration, and further reduces issues for appeal.

With entry of the present amendment claims 5 – 12, 14, 15 and 27 – 40 are pending. New claims have not been added. Claims 1, 3 and 13 have been canceled, and claims 5 – 12, 14, 15 and 27 – 32 have been amended.

In the Office Action dated February 12, 2002, the Examiner indicated claims 9, 10, 12, 31 and 32 were allowable if rewritten in independent form incorporating all the limitations of the base claim and any intervening claims.

Claims 6, 7, 8, 9, 10 and 11 have each been rewritten in independent form. Claims 6, 7 and 8 incorporate the limitations of now canceled claims 1 and 3. Claims 9 and 10 incorporate the limitations of now canceled claim 1. Claim 11 incorporates the limitation of claim 1, and further defines the mature pullulanase as obtainable from *B. deramificans*. This limitation was originally presented in dependent claim 12. Claim 12, which depends from claim 11, has been amended to omit this language.

The dependency of claim 5 has been changed to claim 6.

Claim 14 has been rewritten in independent form incorporating the limitations of claim 6 and presently canceled claim 13. The dependency of claim 15 has been changed to claim 14 as opposed to claim 13.

Claim 27, directed to an enzyme composition, has been rewritten in Markush format incorporating the truncated pullulanases as claimed in claims 6, 7, and 8 and incorporating the limitations of now canceled claims 1 and 3. Minor grammatical changes have been made to dependent claims 28, 29 and 30.

Claims 31 and 32 directed to an enzyme composition comprising a truncated pullulanase have been amended to depend from allowable claims 9 and 10 respectively.

Rejection under 35 U.S.C. §112, second paragraph.

Claims 6 - 8 and 11 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Specifically, the Examiner states there is insufficient antecedent basis for the limitation of "modification" as recited in the claims. The claims have been amended to provide proper antecedent basis for the term modified where appropriate.

Rejections under 35 U.S.C. §102.

Claims 1, 27 and 38 stand rejected under 35 U.S.C. §102(b) as being anticipated by the newly cited reference to Ara et al., (Biosci. Biotech.Biochem). Claim 1 has been cancelled. Claim 27 has been amended to incorporate the limitations of original claims 1 and 3 and to recite in Markush format the truncated pullulanases of claims 6, 7 and 8. Claim 38 depends from claim 27. Cancellation of and amendment of said claims has rendered the rejection moot.

Claims 1, 3, 5, 11, 13 - 15, 27 and 33 - 39 stand rejected under 35 USC §102(e) as being anticipated by Deweer et al. (US 6,074,854). Applicants respectfully traverse the rejection.

The Examiner states,

> "Deweer et al. disclose an identical truncated pullulanase in which the first 29 amino acids have been deleted, or modified by addition of an amino acid to the N-terminal region, isolated from *B. deramificans* T89.117D, produced by a host cell comprising the nucleic acid that has 70% identity to SEQ ID NO: 1 encoding the mature pullulanase, a composition comprising the modified pullulanase further comprising another enzyme such a glucoamylase isolated from *Aspergillus* strains such as *A. niger*, *A. awamori* and *A. foetidus*, a composition which is in solid or liquid form or comprising 60% modified pullulanase."

Applicants assert Deweers et al. is concerned with a full-length pullulanase derived from *B. deramificans* T 89.117D. The mature full length pullulanase has 928 amino acids as shown in SEQ ID NO: 11. At column 5, lines 36 - 65 the reference discloses "[T]he invention also relates to the isolation and provision of a DNA molecule comprising the nucleotide sequence (SEQ ID NO: 10) which codes for the pullulanase of *B. deramificans* T 89.117D (LMG P-13056) or a modified sequence derived therefrom ....." Further it is stated, "[T]he invention also relates to a modified pullulanase, that is to say an enzyme in which the amino acid sequence differs from that of the wild enzyme by at least one amino acid."

Applicants contend the disclosure in Deweers et al. does not teach a truncated pullulanase. For a reference to anticipate Applicants' claimed invention each limitation as claimed must be found in the prior art reference. The Deweers et al. reference fails this test. While arguendo, Deweers et al. disclose that the wild-type pullulanase could be modified, there is no teaching whatsoever, of a truncated pullulanase as claimed in Applicants' application. What Deweers et al. teach is that the pullulanase is synthesized in the form of a precursor protein wherein the signal sequence includes 29 amino acids. Generally signal sequences are eliminated during the exportation of an enzyme to the outside of the cell and is not part of the mature protein.

Rejections under 35 U.S.C. §103.

Claims 1,3, 5 - 8, 11, 13 - 30 and 33 - 40 stand rejected under 35 U.S.C §103(a) as being unpatentable over Deweer et al. (US Patent No. 6,074,854) and McPherson et al. (Biochem. Soc. Trans., 1988, Vol 16(5):723-724) or Albertson et al. (Biochim. Biophys. Acta. 1997, Vol. 1354(1): 35-39). Applicants respectfully traverse this rejection.

Deweer et al. is addressed herein above. As previously stated, the 29 amino acids as disclosed by Deweer et al. is the signal peptide sequence. The removal of the signal peptide to obtain a mature enzyme does not comprise a truncated enzyme as claimed by the Applicants.

The Deweer et al. reference has been combined with McPherson et al. or Albertson et al. As recognized by the Examiner, neither McPherson et al. or Albertson et al. teach a truncated *Bacillus* pullulanase

McPherson et al. teach the modification of deleting about 170 amino acid residues from the amino terminal end of *K. pneumoniae* pullulanase wherein the modification leads to higher activity as compared to the native enzyme (0.81 unit/mg vs 0.58 unit/mg).

Albertson et al. discloses a recombinant plasmid pNZ1452, which includes a 381bp deletion of the 5'region of a *Caldicellulosiruptor saccharolyticus* pullulanase. The plasmid when cloned into *E. coli* produced a pullulanase missing the first 95 amino acid residues but still able to hydrolyze pullulan.

The Examiner states,

> "It would have been obvious to one skilled in the art at the time the invention was made to combine the teaching of Deweer et al. with that of McPherson et al. or Albertson et al. to make a modified pullulanase in which N-terminal amino acids have been deleted or few amino acids are added to the N-terminal end. This is because Deweer et al teach a pullulanase isolated from a *Bacillus*, *B. deramificans*, which is very large enzyme with more than 900 amino acids. McPherson et al. teach a method of increasing the efficiency of large size pullulanase by determining and deleting non-essential amino acids in the N-terminal region and Albertson et al. and McPherson et al. teach that deletion of up to at least 100 - 300 amino acids does not affect the activity of the enzyme negatively but on the other hand increase the efficiency of the enzymes by nearly 30%."

However, Applicants contend the combination of references does not contain a sufficient teaching of how to obtain a truncated pullulanase from a *Bacillus* species wherein the truncated enzyme retains the capacity to hydrolyze alpha-1,6-glucosidic bonds.

As taught at page 8 of Applicants' specification, the deletion in the amino terminal amino acids of a *Bacillus* pullulanase can be of varying length, but is at least three amino acids in

length and the deletion can go no further than the beginning of the first conserved domain which in *B. deramificans* is the tyrosine at amino acid residue 310 as shown in Figure 1. Also as disclosed by the Applicants at page 12 of the specification, Albertson et al. reveal the regions called DPY, A, B, C, D, E, and YNWGY as conserved regions among a group of gram-positive and gram-negative pullulanases. Two regions, DPY and YNWGY were identified as being characteristic of true pullulanases. In addition to the conserved regions highlighted by Albertson et al., Applicants significantly disclose two other conserved regions closer to the N-terminus of pullulanase. These regions are referred to as Y and VWAP and reference is made Figures 2A – 2D of the specification. These regions are not taught or suggested by Albertson et al., as being conserved regions. Applicants further disclose that the limits of amino acid truncations in the N-terminus of pullulanase would not go beyond the Y region.

Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-7620.

Respectfully submitted,

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Appendix I
Version With Markings To Show Changes Made

5.(Twice amended) The pullulanase of **[Claim 3]** **Claim 6,** wherein the *B. deramificans* pullulanase has the designation T89.117D in the LMG culture collection.

6.(Twice amended) **[The pullulanase of Claim 1 wherein the modification is]** **A truncated *Bacillus* pullulanase comprising**

a deletion of **about 100** amino acids from the amino terminus **[of about 100 amino acids]** **of a *Bacillus* pullulanase wherein the *Bacillus* is selected from the group consisting of *B. subtilis, B. deramificans, B. stearothermophilus, B. naganoensis, B. flavocaldarius, B. acidopullulyticus, Bacillus sp* APC-9603, *B. sectorramus, B. cereus,* and *B. fermus* and wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

7.(Twice amended) **[The pullulanase of Claim 1 wherein the modification is]** **A truncated *Bacillus* pullulanase comprising**

a deletion of **about 200** amino acids from the amino terminus **[of about 200 amino acids]** **of a *Bacillus* pullulanase wherein the *Bacillus* is selected from the group consisting of *B. subtilis, B. deramificans, B. stearothermophilus, B. naganoensis, B. flavocaldarius, B. acidopullulyticus, Bacillus sp* APC-9603, *B. sectorramus, B. cereus,* and *B. fermus* and wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

8.(Twice amended) **[The pullulanase of Claim 1 wherein the modification is]** **A truncated *Bacillus* pullulanase comprising**

a deletion of **about 300** amino acids from the amino terminus **[of about 300 amino acids]** **of a *Bacillus* pullulanase wherein the *Bacillus* is selected from the group consisting of *B. subtilis, B. deramificans, B. stearothermophilus, B. naganoensis, B. flavocaldarius, B. acidopullulyticus, Bacillus sp* APC-9603, *B. sectorramus, B. cereus,* and *B. fermus* and wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

9.(Twice amended) **[The pullulanase of Claim 6 wherein the]** **A truncated *Bacillus* pullulanase comprising a** deletion **that** is 98 amino acids from the amino terminus of *Bacillus deramificans* pullulanase**, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

10.(Twice amended) **[The pullulanase of Claim 6 wherein the]** **A truncated *Bacillus* pullulanase comprising a** deletion **that** is 102 amino acids from the amino terminus of *Bacillus deramificans* pullulanase**, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

11.(Twice amended) **[The pullulanase of Claim 1]** **A modified *Bacillus* pullulanase which is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond,** wherein the modification is an addition of at least one amino acid to the amino terminus of the mature pullulanase amino acid sequence **obtainable from *Bacillus deramificans***.

12.(Twice amended) The pullulanase of Claim 11**,** wherein the **[pullulanase is obtainable from *Bacillus deramificans* and the]** additional amino acid at the amino terminus is an Alanine.

14.(Twice Amended) **[The pullulanase of Claim 13 wherein the]** **A truncated *Bacillus* pullulanase produced by a method comprising the steps of**

a) obtaining a recombinant host cell comprising nucleic acid encoding mature pullulanase **[has]** **having** at least 70% identity to the polynucleotide sequence as shown in SEQ ID NO:1,

b) culturing said host cell under conditions suitable for the production of a truncated pullulanase, and

c) recovering the truncated pullulanase

wherein the truncated *Bacillus* pullulanase comprises a deletion of about 100 amino acids from the amino terminus of a *Bacillus* pullulanase wherein the *Bacillus* is selected from the group consisting of *B. subtilis, B. deramificans, B. stearothermophilus, B. naganoensis, B. flavocaldarius, B. acidopullulyticus, Bacillus sp* APC-9603, *B. sectorramus, B. cereus*, and *B. fermus* and said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

15.(Twice amended) The pullulanase of **[Claim 13]** **Claim 14,** wherein the host cell is *B. licheniformis* which comprises a first gene encoding Carlsberg protease and a second gene encoding endo Glu C protease, the first and/or second gene which codes for the protease(s) having been altered such that the protease(s) is/are inactivated.

27.(Twice amended) An enzymatic composition comprising a truncated *Bacillus* pullulanase **wherein said truncated pullulanase is selected from the group of pullulanases consisting of a) a deletion of up to about 100 amino acids from the amino terminus of a *Bacillus* pullulanase, b) a deletion of up to about 200 amino acids from the amino terminus of a *Bacillus* pullulanase, and c)a deletion of up to about 300 amino acids from the amino terminus of a *Bacillus* pullulanase wherein the *Bacillus* is selected from the group consisting of *B. subtilis, B. deramificans, B. stearothermophilus, B. naganoensis, B. flavocaldarius, B. acidopullulyticus, Bacillus sp* APC-9603, *B. sectorramus, B. cereus,* and *B. fermus* and wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond**.

28.(Twice amended) The enzymatic composition of Claim 27, wherein the pullulanase has a deletion of **up to about 100** amino acids from the amino terminus **[of up to about 100 amino acids]**.

29.(Twice amended) The enzymatic composition of Claim 27, wherein the pullulanase has a deletion of **up to about 200** amino acids from the amino terminus **[of up to about 200 amino acids]**.

30.(Twice amended) The enzymatic composition of Claim 27, wherein the pullulanase has a deletion of **up to about 300** amino acids from the amino terminus **[of up to about 300 amino acids]**.

31.(Twice Amended) **[The composition of Claim 27]** **An enzymatic composition comprising the pullulanase of Claim 9,** wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 99, a glutamic acid.

32.(Twice Amended) **[The composition of Claim 27]** **An enzymatic composition comprising the pullulanase of Claim 10,** wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 103, a glutamic acid.